New Test on Tap for Tracking Plum Pox

Tracking the spread of the plum pox potyvirus in stone fruit crops could get a lot easier thanks to a new test devised by Agricultural Research Service scientists.

Plum pox is an aphid-borne disease that causes acidity; pale, concentric rings; and other defects that diminish the eating quality of peaches, pears, plums, and other stone fruits. The disease has devastated the *Prunus* industry in Europe, and USDA is working hard to prevent the same thing from happening here. To that end, ARS researchers Bill Schneider, Reid Frederick, and Vern Damsteegt devised the new test to expedite year-round monitoring of plum pox, particularly in and around Pennsylvania's Adams, Cumberland, Franklin, and York counties—the only U.S. sites where the disease is known to occur.

Each year, Pennsylvania Department of Agriculture survey crews collect thousands of leaf samples from commercial orchards, nurseries, residential properties, and other sites for analysis in diagnostic laboratories. It's a monumental task, but one that's deemed vital to eradicating plum pox as an economic threat to the rest of the state as well as to the nation's $1.8 billion stone fruit industry.

"What makes this virus so hard to detect is that it only shows up at certain times of the growing season and in certain parts of the tree," says Schneider, who, along with Damsteegt and Frederick, works at ARS' Foreign Disease-Weed Science Research Unit, in Fort Detrick, Maryland. "We were looking for a faster, more sensitive method of detecting PPV and chose real-time PCR."

PCR stands for polymerase chain reaction, a chemical procedure that mass-produces copies of particular DNA fragments or genes so they can be identified by other means. With real-time PCR, a targeted fragment becomes detectable virtually the same instant it's mass-produced, or "amplified."

"Plum pox shows up in spring and tapers off by summer. So you're limited to a 1- to 2-month window for effective testing," says Schneider. "By the time the temperature reaches 84˚F–90˚F, the concentration of virus goes down dramatically." When this happens, he continues, standard tests like the ELISA (Enzyme-Linked Immunosorbent Assay) can give misleading results, such as false negatives. Although PCR-based methods are also used, including one called immunocapture PCR, they're labor intensive and time consuming.

"Immunocapture PCR is technically difficult, and if you don't do it right, it can lead to false positives," says Schneider. The ELISA detection of plum pox is based on monoclonal antibodies binding to the virus's coat proteins, while the PCR methods lock onto the gene for making the proteins. Both take about a day to run. Schneider says real-time PCR is faster, simpler, and (unlike ELISA) works well with samples that have been placed in prolonged cold storage—a feature that's especially convenient when laboratories are pressed for time.

"From leaf to final results, real-time PCR takes from 4 to 6 hours, depending on how hard it is to extract viral RNA from a sample," says Schneider. Also, "Real-time PCR is sensitive enough that you can pick up the virus in individual aphids, which is something the other methods don't do."

The added specificity of fluorescent probes used in real-time PCR also enables it to differentiate plum pox's coat protein gene from those of other common *Prunus*-infecting viruses.

"The real-time test is very quantitative," says Schneider, "meaning you can tell how much virus is in a sample," even during hot weather. This information is critical to tracking where, when, and how quickly the virus has spread.—By **Jan Suszkiw,** ARS.

This research is part of Crop Production, Product Value, and Safety, an ARS National Program (#303) described on the World Wide Web at www.nps.ars.usda.gov.

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FRED GILDOW (K10749-1)

Aphids (about 1 mm long) feeding on a peach. These insects are known to spread plum pox on peaches and other stone fruits.

VERN DAMSTEEGT (K10749-2)

Plum pox symptoms on peach leaves.